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Alternatives to the Use of Live Vertebrates in Biomedical Research and Testing

A Bibliography with Abstracts

To Assist In:

- Refining Existing Test Methods
- Reducing Animal Usage
- Replacing Animals As Test Systems

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The Scientific Community, concerned about animal welfare, is sensitive to concerns regarding how and why animals are used in biomedical research and testing to evaluate the toxicological potential of various substances. Although alternatives to methods based on the use of animals may not satisfy all requirements and needs of the biomedical research and toxicologic testing communities, alternatives to the use of vertebrates are being developed and evaluated. Research on such methodologies is aimed at refining procedures to reduce pain and discomfort; reduce the number of animals required to provide scientifically valuable results; and to replace live vertebrates when an alternative methodology can be verified and validated by the scientific community.

The purpose of these bibliographies on "animal alternatives" is to provide a survey of the literature in a format which facilitates easy scanning. This bibliography includes citations from published articles, books, book chapters, and technical reports. Citations to items in non-English languages are indicated with [ ] around the title. The language is also indicated. Citations with abstracts or annotations relating to the method are organized under subject categories. This publication features citations which deal with methods, tests, assays or procedures which may prove useful in establishing alternatives to the use of intact vertebrates. Citations are selected and compiled through searching various computerized on-line bibliographic databases of the National Library of Medicine, National Institutes of Health.

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Suggestions and comments are welcome.

BRAIN/CNS
1
Kobayashi H, Saito F, Yuyama A. EFFECTS OF ORGANOTINS ON THE CHOLINERGIC SYSTEM IN THE CHICKEN BRAIN IN VITRO. Toxicol In Vitro 1992;6(4):337-343.

The effects of two organotins, trimethyltin chloride

(TMT) and tributyltin chloride (TBT), on the cholinergic system in the chicken brain were investigated in vitro. Both compounds, at concentrations below 10-4 M, had almost no effect on acetylcholinesterase activity in cortical homogenate. By contrast,TMT and TBT inhibited non-competitively the activity of choline acetyltransferase with Ki values of 1.50-5. These inhibitory effects were not reversed in the presence of cysteine. TMT and TBT inhibited the low-affinity uptake of choline with Ki values of 2.6. Although both compounds inhibited the depolarized release of acteylcholine (ACh) from slices of brain, only TBT at 10-4 M suppressed the synthesis of ACh. TBT, but not TMT, inhibited the binding of (3H)quinuclidinyl benzilate, which is an inhibitor of muscarinic ACh receptors, at 10-5 and 10-4 M. It is suggested that cholinergic transmission, in particular the synthesis of ACh and the release of ACh, is susceptible to trialkyltin neurotoxicity.

CANCER
2
Hozier J, Applegate M, Moore MM. IN VITRO MAMMALIAN MUTAGENESIS AS A MODEL FOR GENETIC LESIONS IN HUMAN CANCER. Mutat Res 1992;270(2):201-9. (63 REFS)

Recently in vitro assays of mutagenesis have been criticized as being poorly predictive of long-term in vivo rodent assays of carcinogenicity. Questions have also been raised concerning the relevance of rodent assays to human risk. In vitro assays using mammalian cells can detect most types of genetic lesions thought to be important in human malignant disease. Molecular and cytogenetic analyses of mutations induced by a variety of genotoxic compounds at the heterozygous thymidine kinase locus in mouse lymphoma cells indicate that this in vitro assay does indeed register the range of genetic lesions recently found in a wide variety of human tumors. The types and complexity of the induced lesions are reflected in mutant colony phenotype in a compound-specific fashion. These studies point to the use of appropriate in vitro mammalian mutagenesis

assays as new model systems for dissecting the genetic lesions important in human carcinogenesis, and as a means of determining the potential for compounds to induce such lesions.

3
Brown JM, Evans J, Kovacs MS. THE PREDICTION OF HUMAN TUMOR RADIOSENSITIVITY IN SITU: AN APPROACH USING CHROMOSOME ABERRATIONS DETECTED BY FLUORESCENCE IN SITU HYBRIDIZATION. Int J Radiat Oncol Biol Phys 1992; 24(2):279-86.

No method of predicting the radiation sensitivity of individual human tumors is presently available, and recently published data show that other factors, in addition to the intrinsic radiosensitivity of the tumor cells, may play a role in the in vivo response of human tumors. Since these factors likely involve the tumor milieu (e.g., cell-cell contact and tumor hypoxia), an in situ assay of radiosensitivity is required. Although an analysis based on chromosome damage is the only suitable assay that would fit the requirements of sensitivity and speed of analysis, conventional examination of chromosome damage is impractical. By allowing the visualization of chromosomes in interphase cells, the technique of premature chromosome condensation (PCC) overcomes the need to culture the tumor cells in vitro, but the technical problem remains of counting a small excess number of breaks over the often large pretreatment chromosome number. It was demonstrated by the authors that the combination of fluorescence in situ hybridization (FISH) with PCC enormously simplifies the problem by focusing the analysis on a single chromosome. It also allows exchange aberrations to be scored easily. The FISH technology may also be used to estimate radiation sensitivity from stable reciprocal translocations in metaphase identified by combining whole chromosome painting with a second color hybridization to the repeat sequences common to the centromeres. Since the frequency of stable translocations should correlate with initial chromosome damage, and since these translocations are not preferentially lost from the irradiated tumor cell population by cell death, an estimate of tumor cell killing following 1-5 dose fractions should be possible. Each of the two methods has its advantages, and a careful study of the two should establish which is superior for routine use to determine tumor radiosensitivity in situ.

CARCINOGENICITY
4
Li F, Segal A, Solomon JJ. IN VITRO REACTION OF ETHYLENE OXIDE WITH DNA AND CHARACTERIZATION OF DNA ADDUCTS. Chem-Biol Interact 1992;83(1):35-54.

Ethylene oxide (EO) is a direct-acting SN2 alkylating agent and a rodent and probable human carcinogen. In vitro reactions of EO with calf thymus DNA in aqueous solution at neutral pH and 37~ C for 10 h resulted in the following 2-hydroxyethyl (HE) adducts (nmol/mg

DNA): 7-HE-Gua (330), 3-HE-Ade (39), 1-HE-Ade (28), N6-HE-dAdo (6.2), 3-HE-Cyt (3.1), 3-HE-Ura (0.8) and 3-HE-dThd (2.0). Reference(marker) compounds were synthesized from reactions of EO with 2'-deoxyribonucleosides and DNA bases, isolated by paper and high performance liquid chromatography and characterized on the basis of chemical properties and UV, NMR and mass spectra. In agreement with our earlier studies with propylene oxide (PO) (Chem.-Biol. Interact., 67 (1988) 275-294) and glycidol (Cancer Biochem. Biophys., 11 (1990)59-67), alkylation at N-3 of dCyd by EO under physiological conditions resulted in the rapid hydrolytic deamination of 3-HE-dCyd to 3-HE-dUrd. The hydroxyl group on the alkyl side chain which forms after epoxide alkylation is mechanistically involved in this rapid hydrolytic deamination. The results may provide important insights into the mechanisms of mutagenicity and carcinogenicity exhibited by EO and other SN2 aliphatic epoxides.

CELL CULTURE
5
Pesonen M, Andersson T. TOXIC EFFECTS OF BLEACHED AND UNBLEACHED PAPER MILL EFFLUENTS IN PRIMARY CULTURES OF RAINBOW TROUT HEPATOCYTES. Ecotoxicol Environ Saf 1992; 24(1):63-71.

Toxic effects of unbleached (sulfate or sulfite) and bleached (sulfate) paper mill effluents were studied in a primary culture of rainbow trout liver cells. The effluents and control water from a clean area were extracted with diethyl ether and added to the cultures dissolved in dimethyl sulfoxide. Plasma membrane integrity was studied by measuring lactate dehydrogenase (LDH) leakage. The cellular content of glutathione (GSH) was used as an indicator of oxidative stress and the formation of reactive intermediates.

Dose-response studies indicated that unbleached effluents contained more potent toxic substances than bleached effluents. Both unbleached and bleached effluents contained organic diethyl ether-extractable substances which increased cytochrome P450-dependent 7-ethoxyresorufin-O-deethylase (EROD) activities. The inducing effects were seen at concentrations substantially lower than those decreasing GSH content and increasing LDH leakage. At higher concentrations the effluents contained substances that inhibited the cytochrome P450 system. The results show that the trout primary hepatocyte cultures afford a convenient in vitro method for screening cytochrome P450 inducing components extracted from industrial effluents to investigate mechanisms by which wastewaters cause injury in cells.

6
Tashiro T. CELL CULTURE AND ITS APPLICATION--IN VITRO EVALUATION OF ANTICANCER ACTIVITY USING HUMAN TUMOR CELL LINES. Gan To Kagaku Ryoho 1992;19(12):2107-12.

Selective toxicity against cancer cells is a most important determinant for anticancer agents. Therefore, we have preferably evaluated anticancer effects in vivo using murine tumor models for several decades. Approximately 50 anticancer agents are currently available for clinical therapy, but very few agents are effective against some types of cancer. Much progresses in cell culture techniques resulted in establishment of various human tumor cell lines. Currently, we are able to use human tumor lines as well as murine ones for the examination of drug sensitivity. A number of assay methods to evaluate anticancer activity have been developed. In the beginning, growth inhibitory activity was evaluated by counting cell numbers after drug exposure. Then, human tumor clonogenic assay (HTCA) was designed to measure only proliferative cells. Recently colorimetric MTT assay and SRB assay in 96-well microplates were developed, which were adopted in the screening system in the NCI, based on a new idea, that is, disease-oriented screening (DOS) using about 60 human tumor cell lines. In this paper, the outline of each method was described, adding especially several comments on disease-oriented screening.

7
Gille JJ, Joenje H. CELL CULTURE MODELS FOR OXIDATIVE

STRESS: SUPEROXIDE AND HYDROGEN PEROXIDE VERSUS NORMOBARIC HYPEROXIA. Mutat Res 1992;275(3-6):405-14. (80 REFS)

According to the free radical theory of aging, loss of cellular function during aging is a consequence of accumulating subcellular damage inflicted by activated oxygen species. In cells, the deleterious effects of activated oxygen species may become manifest when the balance between radical formation and destruction (removal) is disturbed creating a situation denoted as 'oxidative stress'. Cell culture systems are especially useful to study the effects of oxidative stress, in terms of both toxicity and cellular adaptive responses. A better understanding of such processes may be pertinent to fully comprehend the cellular aging process. This article reviews three model systems for oxidative stress: extracellular sources of O2-. and H2O2, and normobaric hyperoxia (elevated ambient oxygen). Methodological and practical aspects of these exposure models are discussed, as well as the prominent effects observed in cultures of Chinese hamster cell lines. Since chronic exposure models are to be preferred, it is argued that normobaric hyperoxia is particularly relevant oxidative stress model for in vitro cellular aging studies.

8
May JV, Bridge AJ, Gotcher ED, Gangrade BK. THE REGULATION OF PORCINE THECA CELL PROLIFERATION IN VITRO: SYNERGISTIC ACTIONS OF EPIDERMAL GROWTH FACTOR AND PLATELET-DERIVED GROWTH FACTOR. Endocrinology (Baltimore) 1992;131(2):689-97.

An important but poorly understood aspect of mammalian follicle development involves the regulation of theca cell proliferation. To investigate the premise that growth factors regulate theca cell proliferation, porcine theca cells were prepared by collagenase/DN'ase digestion of follicle linings after the removal of the granulosa cells and allowed to attach for 24 hours. This method provided a monolayer of theca cells that had little if any granulosa cell contamination and which secreted high levels of androstenedione relative to granulosa cells during moderate-term culture (33-fold difference). In medium containing fetal calf serum (10%), theca cells were significantly more responsive to platelet-derived growth factor (PDGF) than epidermal growth factor (EGF) in terms of proliferation (13.4- vs. 7.0-fold increase relative to

the initial cell count). This is in contrast to granulosa cells which were significantly more responsive to EGF and PDGF (7.1- vs. 4.0-fold increases). Since serum has been shown to contain both EGF and PDGF, proliferation studies were performed using plasma-derived serum (PDS) which is growth factor restricted to examine more closely the direct effects of growth factors. In medium containing 0.25% PDS and within expts., PDGF (1-25 ng/mL) stimulated theca cell proliferation in a dose-dependent manner (2.3-fold increase relative to controls), whereas EGF did not. EGF, however, markedly enhanced the proliferative action of PDGF (6.4-fold increase relative to controls). Temporal studies in vitro indicate that theca cell proliferation is low during the first 3-day exposure to growth factors irresp. of treatment (a 2-fold increase over the seeding d.). During the second 3-day exposure, however, theca cell proliferation increases 4- to 5-fold. The results of experiments suggest that PDGF is a major mitogen toward porcine theca cells and that EGF greatly enhances its activity. Such results coupled with those demonstrating PDGF enhancement of EGF/TGF-alpha-stimulated granulosa cell proliferation suggest that these two growth factors may act directly and synergistically to promote proliferation of both theca and granulosa cells leading to follicle growth.

9
Farage-Elawar M, Rowles TK. TOXICOLOGY OF CARBARYL AND ALDICARB ON BRAIN AND LIMB CULTURES OF CHICK EMBRYOS. J Appl Toxicol;12(4):239-44.

Chick embryo brain and limb bud cultures were treated with different concentrations of either carbaryl or aldicarb with or without activation (.+-.S-9) for 5 days. Viability and cytotoxicity using the neutral red assay, and carbamate effects on cell migration and colony spread were measured. S-9 decreased the effects of carbaryl and aldicarb on brain cell cytotoxicity at exposures of 15-60 and 40-200 ppm, respectively, as indicated by increased concentrations of neutral red. Viability of brain cell cultures was not altered by aldicarb, but was decreased by carbaryl plus S-9 in concentrations of >40 ppm. In limb cultures, carbaryl without S-9 was significantly toxic at 8-25 ppm, but only concentrations of >25 ppm of carbaryl plus S-9 significantly affected cytotoxicity. In contrast, aldicarb without S-9 caused no effect on limb cell cytotoxicity at concentrations of 40-200 ppm, but aldicarb plus S-9 significantly reduced cellular

cytotoxicity at concentrations of >160 ppm. Carbaryl .+-.S-9 decreased the spread of both brain and limb colonies; aldicarb .+-.S-9 caused a significant increase in the spread of the brain but not limb colonies. In summary, toxicities of carbaryl and aldicarb can be detected in vitro, but these 2 carbamates have a selective toxicity, with carbaryl toxic to brain and limb buds and aldicarb more toxic to brain than limb cells. S-9 was more likely to decrease the toxicity of carbaryl than the toxicity of aldicarb.

10
Cascales M, Martin-Sanz P, Alvarez A, Sanchez-Perez M, Diez Fernandez C, Bosca L. ISOENZYMES OF CARBOHYDRATE METABOLISM IN PRIMARY CULTURES OF HEPATOCYTES FROM THIOACETAMIDE-INDUCED RAT LIVER NECROSIS: RESPONSES TO GROWTH FACTORS. Hepatology 1992;16(1):232-240.

Hepatocytes isolated from the liver of rats after a necrotizing dose of thioacetamide (6.6 mmol/kg) were used to study the postnecrotic process of liver regeneration. Flow cytometry analysis revealed populations of dedifferentiated hepatocytes exhibiting physical properties (size and fluorescence emission at 530 nm) similar to those found in fetal (22 days old) liver cells. The percentage of these cells increased progressively from 24 to 48 and 72 hr after thioacetamide administration. In primary cultures of hepatocytes the effects of phorbol-12-myristate-13-acetate, bombesin and insulin were investigated on the 6-phosphofructo-2-kinase/fructose-2, 6-bisphosphate system. Bombesin and insulin stimulated 6-phosphofructo-2-kinase activity and fructose-2, 6-bisphosphate content both in control and in thioacetamide-treated hepatocytes. However, phorbol-12-myristate-13-acetate stimulated 6-phosphofructo-2-kinase activity and increased fructose-2, 6-bisphosphate concentration in thioacetamide-treated liver cells, whereas no similar response was found in hepatocytes from control rats. The response of postnecroticthioacetamide-treated hepatocytes to phorbol-12-myristate-13-acetate was similar to that obtained from 22-day-old fetal liver cells, which reveals that different methods might control fructose 2,6-bisphosphate content and therefore the mechanisms of glycolysis and gluconeogenesis at this regulatory step. The isoenzyme pattern of hexokinases found elicits a complete disturbance in glucokinase and hexokinases activities. These results led the authors to conclude that the characteristics and behavior of

thioacetamide-induced postnecrotic hepatocytes are closely related to those found in fetal liver cells.

11
Chan TC, Boon GD, Shaffer L, Redmond R. ANTIVIRAL NUCLEOSIDE TOXICITY IN CANINE BONE MARROW PROGENITOR CELLS AND ITS RELATIONSHIP TO DRUG PERMEATION. Eur J Haematol;49(2):71-6.

The most promising nucleoside analogs that are currently undergoing preclinical and clinical testing for anti-HIV activity belong to the dideoxynucleoside group. We have studied the toxicity of 3'-azido,3'-deoxythymidine (AZT), 2',3'-dideoxycytidine (DDC), and 2',3'-dideoxyinosine (DDI) in canine bone marrow progenitor cells in culture. AZT potently inhibited both canine CFU-GM and CFU-E with IC50 values of 2 and 8 mumol/l respectively, while DDC was relatively non-toxic to either progenitor with IC50 of > 200 mumol/l and 80 mumol/l respectively. DDI was mildly toxic to the bone marrow progenitors, with IC50 values of 62 mumol/l for CFU-GM and 70 mumol/l for CFU-E. Dipyridamole, a nucleoside transport inhibitor, did not influence the toxicity of these dideoxynucleosides in either progenitor at concentrations up to 10 mumol/l. Permeation of radiolabeled AZT into bone marrow mononuclear cells was slow and non-saturable, while the permeation of DDI was even slower. DDC did not permeate bone marrow cells well, with very little cell accumulation even after 2 hours of equilibration. Our toxicity data from canine bone marrow progenitor cells paralleled the clinical hematotoxicity profiles of these dideoxynucleosides in AIDS patients and suggest that the myelotoxicity of a nucleoside analog is related to its ability to permeate the progenitor cells in question. Canine bone marrow progenitor cultures may serve well as an in vitro model for drug hematotoxicity studies.

12
Dou M, de Sousa G, Lacarelle B, Placidi M, Lechene de la Porte P, Domingo M, Lafont H, Rahmani R. THAWED HUMAN HEPATOCYTES IN PRIMARY CULTURE. Cryobiology 1992;29(4):454-69.

In drug metabolism studies, isolated and cultured human hepatocytes provide a useful model for overcoming the difficulty of extrapolating from animal data. In vitro studies with human hepatocytes are scarce because of

the lack of livers and suitable methods of storage. After developing a new method for cryo-preservation of human hepatocytes, we evaluated the effects of deep freezing storage on their viability, morphology, and functional and toxicological capabilities in classical culture conditions. Freshly isolated human hepatocytes were cryopreserved in medium containing 10% Me2SO and 20% fetal calf serum, using a Nicool ST20 programmable freezer (-1.9 degrees C/min for 18 min and -30 degrees C/min for 4 min). Cells were stored in liquid nitrogen. Viability of thawed human hepatocytes was 50-65% as assessed by erythrosin exclusion test prior to purification on a Percoll density gradient. Morphological criteria showed that thawed human hepatocytes require an adaptation period to the medium after seeding. Functional assessments showed human hepatocytes that survive freezing and thawing preserve their protein synthesis capabilities and are able to secrete a specific protein, anionic peptidic fraction, which is involved in the hepatic uptake of bile-destined cholesterol. All of the experiments indicated that thawed human hepatocytes should be used 38 h after seeding for optimum recovery of their functions: membrane integrity, protein synthesis, and stabilization of drug metabolism enzymes.

13
Norppa H, Jarventaus H. INDUCTION OF SISTER-CHROMATID EXCHANGES BY 2-AMINOFLUORENE IN CULTURED HUMAN LYMPHOCYTES WITH AND WITHOUT ERYTHROCYTES. Mutat Res 1992;282(3):135-138.

2-Aminofluorene (2-AF), an indirect mutagen reported to be metabolically activated by erythrocytes in the Salmonella mutagenicity test, was studied for the induction of sister-chromatid exchanges (SCEs) in human lymphocytes in vitro with (whole-blood cultures) and without erythrocytes (isolated lymphocytes cultures). 2-AF (0.025-0.8 mM) was prresent in the cultures for the last 48 h of 72-h cultures. In both types of culture, SCEs increased in a dose-dependent manner, with a statistically significant elevation already at the lowest concentration of 2-AF tested and maximum responses of 2.4-fold (whole blood) and 2.1-fold (isolated lymphocytes), in comparison with mean SCEs/cell in control cultures, as 0.4 and 0.2 mM concentrations (respectively). Thus, the induction of SCEs by 2-AF was not dependent on the presence of erythrocytes. The results suggest that leukocytes, but not erythrocytes, are important in the metabolic

activation of 2-AF in the human lymphocyte SCE assay.

14
Amacher DE, Stadler J, Schomaker SJ, Verseil C. POSSIBLE DEVELOPMENTAL EFFECTS OF SOME ARYL TRIAZINE ANTICOCCIDIAL AGENTS IN RAT LIMB BUD MICROMASS CULTURES AND IN RAT EMBRYOCULTURE. 20TH Annual Conference of the European Teratology Society, Wuerzburg, Germany, August 31-September 3, 1992. Teratology 1992;46(3):19A.

No abstract.

CYTOTOXICITY
15
Barlean L, Danila I, Avram G, Cotor F, Durnea C. THE USE OF CELL CULTURES IN THE CYTOTOXICITY TESTING OF DENTAL MATERIALS. Rev Med Chir Soc Med Nat Iasi 1992; 96(1-2):111-3.

On lines of human (HeLa) and monkey (BS-C-1) cell cultures, the cytotoxicity of 15 products of dental use for radicular and coronary fillings, alloys and endodontic antiseptics was analysed. It was found that the simplified method can be used, together with the in vivo and in vitro tests recommended by I.S.O., for determining the bioavailability of dental products.

16
Thoren SA. CALORIMETRY: A NEW QUANTITATIVE IN VITRO METHOD IN CELL TOXICOLOGY: A DOSE/EFFECT STUDY OF ALVEOLAR MACROPHAGES EXPOSED TO PARTICLES. J Toxicol Environ Health 1992;36(4):307-318.

A short-term toxicological test has been developed using a calorimetric method. The metabolic activity, observed as the heat exchange rate, was monitored from alveolar rabbit macrophages in monolayers exposed to different metal and non-metal particles. Calorimetric activity indices and viability indices were Introduced, from which toxic effects could be assessed. Manganese dioxide particles were found to be cytotoxic. In contrast, titanium dioxide particles seemed to be harmless. The results were in accordance with the cell survival found by use of a fluorescein ester staining method and measured by an image analyzer. Toxic effects from quartz in the form of increased metabolic activity of exposed cells could be detected by the calorimeter in contradiction to the use of the image analyzer. This

latter result supports the hypothesis that silica particles cause chronic modification of the macrophage function and that this change in the alveolar macrophage function may be the first of a series of processes leading to pulmonary fibrosis.

17
Van Noorden C JF. ASSESSMENT OF LYSOSOMAL FUNCTION BY QUANTITATIVE HISTOCHEMICAL AND CYTOCHEMICAL METHODS. Histochem J 1991;23(10):429-435.

Quantitative histochemistry and cytochemistry enables a direct link to be made between metabolic functions such as the activity of lysosomal enzymes and the morphology of a tissue or a type of cell. Several approaches exist such as microchemistry based on (bio)chemical analysis of a single cell or a small piece of tissue dissected from a freeze-dried section. Other approaches are cytofluorometry or cytophotometry, which are based on the principle that a fluorescent or coloured final reaction product is precipitated at the site of the enzyme. The amount of final reaction product is analysed per cell or per unit volume of tissue using either a microscope cytofluorometer or flow cytometer for fluorescence measurements of an image analysing system or scanning and integrating cytophotometer for absorbance measurements. In principle, fluorescence methods are to be preferred over chromogenic methods because they are more sensitive and enable multiparameter analysis. However, only limited number of fluorogenic methods are at hand that give a final reaction product which is sufficiently water-insoluble to guarantee good localisation. Chromogenic methods are far better with respect to localisation properties and, therefore, most commonly used for quantitative histochemical analysis of lysosomal enzyme activities. Besides the measurement of enzyme reactions in tissues and cells, chromogenic methods have been applied for the analysis of kinetic parameters of lysosomal enzymes in situ which could be a better reflection of enzymes kinetics in vivo than those obtained in vitro with biochemical means in diluted solutions. This paper reviews briefly fundamental aspects and applications of quantitative histochemical methods in the study of lysosomes.

18
Geara FB, Peters LJ, Ang KK, Wike JL, Brock WA. RADIOSENSITIVITY MEASUREMENT OF KERATINOCYTES AND

FIBROBLASTS FROM RADIOTHERAPY PATIENTS. Int J Radiat Oncol Biol Phys 1992;24(2):287-93.

Genetic diversity is believed to influence cellular radiosensitivity and individual variability in normal tissue reactions to radiotherapy. To measure normal cell radiosensitivity in vitro, we investigated a culture technique that yields keratinocyte and fibroblast cell cultures from small skin biopsy samples (average weight 32 mg). This technique uses 3T3 NIH cells as feeder cells, culture medium containing dialyzed fetal calf serum, low calcium, and various growth factors for keratinocyte growth. A calcium concentration of 4 x 10(-3) M and the use of lethally irradiated NIH 3T3 feeder cells were critical to the success of this method. Primary keratinocyte cultures were successfully obtained from nine biopsy specimens, and radiosensitivity measurements were obtained in six of the resulting strains. Keratinocytes were, in general, more radioresistant than fibroblasts derived from the same specimen. It was conclude that radiosensitivity assessment of keratinocyte and fibroblast cultures derived from small punch biopsy specimens is feasible. Additional studies can now be carried out to determine the degree of variability between individuals and the relationship between in vitro keratinocyte and fibroblast radiosensitivity and their value in predicting normal tissue responses to radiotherapy.

19
Kunimoto M, Aoki Y, Shibata K, Miura T. DIFFERENTIAL CYTOTOXIC EFFECTS OF METHYLMERCURY AND ORGANOTIN COMPOUNDS ON MATURE AND IMMATURE NEURONAL CELLS AND NON-NEURONAL CELLS IN VITRO. Toxicol In Vitro 1992; 6(4):349-355.

The neurotoxicity of methylmercury and organotin compounds was evaluated in vitro on the basis of their differential cytotoxic effects on neuronal cells (rat phaeochromocytoma cell PC12h, human neuroblastoma cell NB-1 and primary cultures of rat cerebellar cells) and non-neuronal cells (normal rat kidney epithelial cell NRK-52E and primary cultures of rat hepatocytes). In this system, neuronal cells show consistently higher sensitivity to the toxicity of methylmercury, trimethyltin and triethyltin, which are known to be neurotoxic, than non-neuronal cells, as judged by the 50% lethal concentrations (LC50) of these compounds after 48 hr treatment. Tributyltin and triphenyltin,

which have not yet been confirmed to be neurotoxic, also show higher toxicity to neuronal cells; their LC50 values for neuronal cells are generaly lower than those for non-neuronal cells, suggesting that they could be neurotoxic. Differential cytotoxic effects of these compounds on mature and immature neuronal cells were also investigated using nerve growth factor (NGF)-treated PC12h and cerebellar cells precultured for 15 days in vitro as mature neuronal cells. This assessment system for neurotoxic compounds based on differential cytotoxicity to neuronal and non-neuronal cells may be useful as a primary screening system for the neurotoxicity of environmental contaminants.

20
Sugimoto Y, Ohe Y, Nishio K, Ohmori T, Fujiwara Y, Saijo N. IN VITRO ENHANCEMENT OF FLUOROPYRIMIDINE-INDUCED CYTOTOXICITY BY LEUCOVORIN IN COLORECTAL AND GASTRIC CARCINOMA CELL LINES BUT NOT IN NON-SMALL-CELL LUNG CARCINOMA CELL LINES. Cancer Chemother Pharmacol 1992;30(6):417-22.

Leucovorin (LV) increases the cytotoxic effect of fluorouracil (FUra) and 5-fluoro-2'-deoxyuridine (FdUrd) by enhancing the formation of the fluorodeoxyuridine monophosphate (FdUMP) thymidylate synthase (TS) 5,10-methylenetetrahydrofolate (mTHF) ternary complex. To study the difference in the efficacy of this combination against different tumors, we compared the effect of LV (20 microM) on the cytotoxicity of FUra, FdUrd, and 5-fluorouridine (FUrd) in vitro against cell lines of five colorectal carcinomas (CC), five gastric carcinomas (GC), and four non-small-cell lung carcinomas (NSCLC) using the colony-forming assay. The in vitro data corresponded well to the results of clinical trials. Therefore, the colony-forming assay may be useful for the identification of the sensitivity of tumors according to phenotype.

21
Wataha JC, Craig RG, Hanks CT. PRECISION OF AND NEW METHODS FOR TESTING IN VITRO ALLOY CYTOTOXICITY. Dent Mater 1992;8(1):65-70.

Previous studies have utilized in vitro alloy cytotoxicity tests to evaluate dental casting alloys. The purposes of this study were to: (1) evaluate the precision of the optical density and visual tests

previously used, (2) evaluate a new test measuring absorbance of solubilized formazan dyes, and (3) test the correlation between these tests for cytotoxicity. Balb/c 3T3 cells were plated in 24-well culture trays at 25,000 cells/cm2 around ten types of dental casting alloys (six samples/alloy) and incubated for 72 h. Cells were histochemically stained with MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide)/succinate for 2 hours, then fixed, washed, and dried. Toxicity was measured by optical densitometer (OD) scanning, visual assessment, and 560-nm absorbance of DMSO-solubilized dyes. Measurements of rings of inhibition were not used, because they did not provide precise data, and correlated poorly with the other methods. The results were analyzed by ANOVA, Tukey intervals, and coefficients of variation (CV's). Results showed that all three methods ranked alloy toxicities similarly ($p = 0.05$). The solubilization method was most discriminating due to lower CV's. Correlation between densitometer and solubilization methods was excellent ($R2 = 0.96$). Between-experiment CV's were generally less than 20%, and often less than 10%. Between-sample CV's were generally less than 20%.

DENTAL TOXICITY
22
McNamara JR, Heithersay GS, Wiebkin OW. CELL RESPONSES TO HYDRON BY A NEW IN-VITRO METHOD. Int Endod J 1992; 25(4):205-12.

An in-vitro biotoxicity test system, suitable for the assessment of endodontic filling materials, has been developed and used to test cell responses to Hydron, AH26 and Tubliseal. A robust, well-characterized and stable cell line (L-cells) which was grown as uniform cultures on Millipore filters, has been used as indicator cells. As they approached confluence they were exposed to test substances for 24 h and biosynthetic activities were measured. The test system is a modification of that described by Wennberg et al. (1979). Prepolymerized Hydron decreased cell functions by 59% and 56% of live cell controls, respectively, while the freshly mixed polymerizing Hydron inhibited biosynthesis by 89% and 94%, respectively. The data for polymerizing Hydron were compared with results for other root-filling materials and showed similar values to those for Tubliseal (92% and 95%), but greater inhibition of biosynthesis than for AH26 (53% and 50%). The AH26 values were similar to those obtained from cultures exposed to the prepolymerized Hydron. Recovery

of biosynthetic capacity by these cultures after removal of all endodontic material was also assessed. Partial biosynthetic recovery of cell cultures was observed 24 h after removal of prepolymerized Hydron.

23
Pissiotis E, Spangberg L. DENTIN AS INHIBITOR OF BACTERIAL TOXICITY ON PULPAL CELLS IN VITRO. J Endod 1992;18(4):166-71.

Many studies have shown that the major cause of pulpal disease is the presence of bacteria or their by-products in the dentinal tubules. The purpose of this investigation was to develop an in vitro model, simulating the pulp chamber, that would permit the study of the transport of bacterial by-products through dentin and their effect on pulpal cells. Human pulpal cells were cultured in a modified Sykes-Moore chamber and exposed through dentin to sonicated extracts of Porphyromonas gingivalis ATCC 33277. The cell response was evaluated with the thymidine incorporation method. The results were compared with the cell response obtained after direct exposure to the same irritant. It was found that dentin significantly restricts the diffusion of bacterial proteins in a 24-h experimental period. The proposed model has further applications in biocompatibility and microleakage research.

24
Barlean L, Danila I, Avram G, Cotor F, Durnea C. THE USE OF CELL CULTURES IN THE CYTOTOXICITY TESTING OF DENTAL MATERIALS. Rev Med Chir Soc Med Nat Iasi 1992; 96(1-2):111-3.

On lines of human (HeLa) and monkey (BS-C-1) cell cultures, the cytotoxicity of 15 products of dental use for radicular and coronary fillings, alloys and endodontic antiseptics was analysed. It was found that the simplified method can be used, together with the in vivo and in vitro tests recommended by I.S.O., for determining the bioavailability of dental products.

25
Wataha JC, Craig RG, Hanks CT. THE EFFECTS OF CLEANING ON THE KINETICS OF IN VITRO METAL RELEASE FROM DENTAL CASTING ALLOYS. J Dent Res 1992;71(7):1417-1422.

The kinetics of the release of elements from six dental

casting alloys into cell-culture medium was assessed by means of atomic absorption spectroscopy. Alloys were evaluated in the polished and polished-cleaned conditions so that the effects of cleaning could be determined. Auger scanning microscopy was used for analysis of the surfaces of selected alloys before and after exposure to the cell-culture medium. Release patterns for each element were characterized by the shape of the dissolution vs. time curve, concentration of the element at 12 h as a percentage of the 72-hour concentration, and the relative slope of the curve from 48 to 72 h. Three patterns of release were observed for elements in these alloys. Cleaning did not change the pattern of release but did generally significantly decrease the quantities of elements released ($p = 0.05$). The type of dissolution vs. time curve appeared to be dependent upon the element and the composition of the alloy. When cleaning reduced dissolution, surface analyses showed that the cleaning process increased the abundance of elements such as Au and Pd and reduced the abundance of Ag and Cu. Elements which were released from the alloys were more abundant on the surface than in the bulk in both polished and polished-cleaned conditions. Auger analyses of alloy surfaces after exposure to medium showed the presence of organic films up to 50 nm thick. This study demonstrated the importance of consideration of the cleaning method and kinetic release pattern when in vitro tests which assess the cytotoxicities of these alloys are planned.

DERMAL
26
Dick IP, Scott RC. PIG EAR SKIN AS AN IN VITRO MODEL FOR HUMAN SKIN PERMEABILITY. J Pharm Pharmacol 1992; 44(8):640-5.

Pig skin has been shown to have similar histological and physiological properties to human skin and has been suggested as a good model for human skin permeability. In this series of experiments, the in-vitro permeability of pig ear skin was compared with human (abdominal) skin and rat (dorsal) skin using both hydrophilic (water, mannitol, paraquat) and lipophilic (aldrin, carbaryl, fluazifop-butyl) penetrants. Pig skin was found to have a closer permeability characteristic than rat skin to human skin, particularly for lipophilic penetrants. Elecectrical conductance measurements across pig skin membranes showed that skin conductance could be a useful method for assessing the integrity of membranes, particularly

when used in conjunction with water permeability assessments.

27
Scott RC, Clowes HM. IN VITRO PERCUTANEOUS ABSORPTION EXPERIMENTS: A GUIDE TO THE TECHNIQUE FOR USE IN TOXICOLOGY ASSESSMENTS. Toxicol Methods 1992; 2(2):113-23.

This article discusses the essential features of the in vitro percutaneous absorption method. Specifically, the design of diffusion cells, choice of suitable receptor fluids, preparation of skin membranes, and the effect of storage and temperature in these experiments are discussed. Literature references that indicate that in vitro assessments of percutaneous absorption can predict in vivo absorption are briefly discussed.

28
Scholes EW, Basketter DA, Lovell WW, Sarll AE, Pendlington RU. THE IDENTIFICATION OF PHOTOALLERGIC POTENTIAL IN THE LOCAL LYMPH NODE ASSAY. Photodermatol Photoimmunol Photomed 1991;8(6):249-254.

Guinea pig test methods are the most commonly used and reliable of predictive models for contact photoallergenicity of chemicals. The murine local lymph node assay (LLNA) has been developed recently as an alternative method for the identification of skin-sensitizing chemicals. Sensitization potential is measured from an assessment of the proliferation of lymphocytes in lymph nodes draining the site of exposure to the test chemical. This work investigates the activity of 6 widely reported photoactive chemicals in a modified LLNA (a photo-LLNA). The photoallergens tetrachlorosalicylanilide and fentichlor elicited positive ultraviolet radiation (UV)-dependent proliferative responses that were greater than their positive UV-independent responses, suggesting that they are both contact and photoallergic in the mouse. The lack of a proliferative response to 6-methylcoumarin and the absence of a reproducible response to musk ambrette suggest that the assay is insufficiently sensitive to identify weak photoallergic potential. The results demonstrate that the photo-LLNA is able to detect at least moderate photoallergic potential.

29

Metcalf SP, Alexander PD, Blackham A, Gibson M. IN VITRO AND IN VIVO METHODS TO EVALUATE PERCUTANEOUS ABSORPTION OF A NEW 5-LIPOXYGENASE INHIBITOR FROM TOPICAL DOSAGE FORMS. Predict Percutaneous Penetration 1991; 113-22.

A synthetic aminopyrazole derivative (APD, not further defined) is being developed as a 5-lipoxygenase inhibitor for the treatment of psoriasis. This paper describes in-vitro studies to evaluate and optimize APD delivery to the skin from topical formulations; an in-vivo model of inflammation was developed to gain further evidence that APD formulations were effective in delivering the drug to elicit an anti-inflammatory response in the skin.

DERMAL TOXICITY

30

Gay R, Swiderek M, Nelson D, Ernesti A. THE LIVING SKIN EQUIVALENT AS A MODEL IN VITRO FOR RANKING THE TOXIC POTENTIAL OF DERMAL IRRITANTS. Toxicol In Vitro 1992;6(4):303-315.

The living skin equivalent (LSE) is an organotypic co-culture composed of human dermal fibroblasts in a collagen-containing matrix overlaid with human keratinocytes that have formed a stratified epidermis. This model system was used as mermatotoxicty model in vitro for studying the effects of test samples topically applied to the air-exposed epidermis. Using the colorimetric thiazolyl blue MTT) conversion assay as a measure of mitochondrial function, the extent of cytotoxicity induced by several well-characterized chemical irritants was evaluated in the LSE. For the seven chemical irritants was evaluated in the LSE. For the seven chemical irritants tested, the concentrations that inhibited MTT conversion by 50% were approximately those threshold concentrations at which irritation was seen in human skin. In addition, nine chemicals that were classified as non-irritating to human skin, including solids and water-insoluble substances, exhibited minimal or no inhibition of MTT conversion when tested at full strength. The data suggest that organotypic skin cultures can be used as model systems for studying certain aspects of chemically induced dermal irritation. Using rates of water penetration as the most meaningful assessment of barrier competence, the LSE is approximately 30-fold more permeable than human skin. Although incomplete as a percutaneous absorption model, the presence of this partial barrier

does influence the responses of cells in the LSE to topically applied chemicals.

31
Kemppainen BW, Mehta M, Stafford R, Riley RT. EFFECT OF VEHICLE ON SKIN PENETRATION AND RETENTION OF A LIPOPHILIC RED TIDE TOXIN (PBTX-3). Toxicon 1992;30(8):931-935.

The purpose of this study was to determine the effect of carrier vehicle on the penetration of brevetoxin ((3H)PbTx-3) into skin layers and receptor fluid. Disks of guinea-pig skin were mounted on penetration chambers. Epidermal surfaces were dosed with 0.320 mug/cm2 of PbTx-3 dissolved in 50 mul of vehicle (water, methanol, or dimethylsulfoxide (DMSO)). In vitro skin penetration by PbTx-3 during 24 hr of exposure was 6.2, 2.3 and 26% for water, methanol and DMSO, respectively (expressed as % of dose applied).

32
Wallace KA, Harbell JW, Accomando N, Triana A, Valone S, Curren RD. EVALUATION OF THE HUMAN EPIDERMAL KERATINOCYTE NEUTRAL RED RELEASE AND NEUTRAL RED UPTAKE ASSAY USING THE FIRST 10 MEIC TEST MATERIALS. Toxicol in Vitro 1992;6(4):367-71.

Two methodologies used in vitro to estimate cytotoxicity in cell culture systems were compared: these were the neutral red uptake assay (NRU), which is used to measure toxicity caused by an extended (48-h) exposure to the test material, and the neutral red release assay (NRR), which is used to measure toxicity caused by a short-term (1-min) exposure to the test material. Both methodologies used the normal human epidermal keratinocyte (NHEK)-based Neutral Red Bioassay supplied by Clonetics Corporation (San Diego, CA, USA). 10 Materials (paracetamol, acetylsalicylic acid, ferrous sulfate, diazepam, amitriptyline, digoxin, ethylene glycol, methanol, ethanol and isopropanol), which are part of the Multicenter Evaluation of In vitro Cytotoxicity (MEIC) panel, were tested. When compared with documented values for either the human acute oral LD or the human acute lethal blood concentration, the NRU assay was much more useful in predicting human acute toxicity than the NRR assay.

33
Fisher HL, Hall LL, Sumler MR, Shah PV. DERMAL PENETRATION OF [14C]CAPTAN IN YOUNG AND ADULT RATS. J Toxicol Environ Health 1992;36(3):251-71.

Age dependence in dermal absorption has been a major concern in risk assessment. Captan, a chloroalkyl thio heterocyclic fungicide, was selected for study of age dependence as representative of this class of pesticides. Dermal penetration of [14C]captan applied at 0.286 mumol/cm2 was determined in young (33-d-old) and adult (82-d-old) female Fischer 344 rats in vivo and by two in vitro methods. Dermal penetration in vivo at 72 hours was about 9% of the recovered dose in both young and adult rats. Two in vitro methods gave variable thermal penetration values compared with in vivo results. A static system yielded 2-fold higher dermal penetration values compared with in vivo results for both young and adult rats. A flow system yielded higher dermal penetration values in young rats and lower penetration values in adults compared with in vivo results. A physiological pharmacokinetic model was developed having a dual compartment for the treated skin and appeared to describe dermal absorption and disposition well. From this model, tissue/blood ratios of captan-derived radioactivity for organs ranged 0.35-3.4, indicating no large uptake or binding preferences by any organ. This preliminary pharmacokinetic model summarizes the experimental findings and could provide impetus for more complex and realistic models.

34
Harvell J, Bason MM, Maibach HI. IN VITRO SKIN IRRITATION ASSAYS: RELEVANCE TO HUMAN SKIN. J Toxicol Clin Toxicol 1992;30(3):359-69. (30 REFS)

No abstract.

35
Ng KM E, Chu I, Bronaugh RL, Franklin CA, Somers DA. PERCUTANEOUS ABSORPTION AND METABOLISM OF PYRENE, BENZO[A]PYRENE, AND BIS(2-ETHYLHEXYL) PHTHALATE: COMPARISON OF IN VITRO AND IN VIVO RESULTS IN THE HAIRLESS GUINEA PIG. Toxicol Appl Pharmacol 1992; 115(2):216-23.

The in vitro and in vivo absorption and metabolism of pyrene, benzo[a]pyrene, and DEHP were investigated in

the hairless guinea pig. The in vitro method, which involved the use of flow-through diffusion cells and Hepes-buffered Hanks' balanced salt solution containing 4% bovine serum albumin as perfusate, was demonstrated to be a suitable system for predicting in vivo absorption of the above lipophilic compounds. The successful application of the in vitro technique for these compounds is significant because no satisfactory in vitro method has hitherto been developed to predict in vivo absorption of highly lipophilic chemicals. Quantification of parent compounds and metabolites that permeated into perfusates and those that remained in skin disks provided insight into the process by which the chemicals penetrated through the skin. Pyrene was absorbed primarily by a passive diffusion process, although a small fraction of the administered dose was biotransformed into metabolites in the skin and partitioned into the receptor fluid. Absorption of benzo[a]pyrene was mediated by biotransformation processes. Data from the present study led to the conclusion that the in vitro method can be utilized to predict in vivo absorption for compounds of high lipophilicity and that dermal metabolism facilitates partitioning of metabolites into the receptor fluid and hence may affect the biological activities of dermally applied compounds.

EMBRYOTOXICITY

36
Noda Y. EVALUATION OF ENVIRONMENTAL FACTORS AFFECTING EMBRYO DEVELOPMENT IN VITRO. Nippon Sanka Fujinka Gakkai Zasshi 1992;44(8):960-70.

Human in vitro fertilization and embryo transfer (IVF-ET) became an indispensable modality for treating infertile patients. Unfortunately, the success rates are not satisfactory in the majority of clinics in the 14 years since the first report of a test tube in 1978. One major issue should be the technique for embryo culture because, in general, mammalian embryos, including humans', are known to exhibit developmental retardation in vitro. In a significant number of embryos, cleavage is arrested at the first or second cell cycle when cultured under the conventional culture conditions. This phenomenon in rodents is known as "block to development in vitro" or "two-cell block in vitro". Recently, the mouse two-cell block was found to be attenuated by the addition of superoxide dismutase (SOD) to the culture medium. SOD is the enzyme that catalyzes the dismutation reaction of superoxide anion

radicals: 2O2- + 2H(+)----H2O2 + O2. This suggests that developmental retardation in vitro may be related to the potential oxygen toxicity that embryos encounter in vitro. By the addition of chemicals to the culture medium such as L-Cysteine, L-Ascorbic acid, EDTA, DTPA or thiredoxine, blastulation rates could be increased overcoming blocking phenomenon. From these findings, it seemed possible to hypothesize that developmental retardation is caused by the oxidative stress that embryos encounter in vitro. To validate the hypothesis, intracellular generation of active oxygen species was measured by using DCHF-DA, a fluorescence dye precursor. The results showed that the fluorescent emissions of embryos were lowest in embryos cultured under 5% O2 and highest under 40% O2. L-Cysteine and thioredoxin, both of which have been shown to promote the embryo development, decreased the fluorescence emissions of embryos.

GENOTOXICITY

37

Hellmer L, Bolcsfoldi G. AN EVALUATION OF THE E. COLI K-12 UVRB/RECA DNA REPAIR HOST-MEDIATED ASSAY. II. IN VIVO RESULTS FOR 36 COMPOUNDS TESTED IN THE MOUSE. Mutat Res 1992;272(2):161-73.

The aim of this study was to further evaluate the E. coli K-12 DNA repair host-mediated assay, as a short-term in vivo genotoxicity test, to be used as a complement to the micronucleus test in the routine testing of chemicals and drugs. The assay involves the administration of the test substance to mice by the route of choice, followed by the intravenous administration of a mixture of DNA repair deficient and proficient derivatives of E. coli K-12. After an incubation period the relative survival of the two strains was determined in blood, liver, lungs, kidneys and testes of the host. A significant preferential reduction of the DNA repair deficient strain in any organ indicates that the test substance possesses genotoxic properties. A total of 36 substances, 26 carcinogens, 4 weak or non-carcinogens and 6 unclassified substances, were tested in this assay. Positive results were obtained for 23 compounds. Of the carcinogens 18 were positive andof the non-carcinogens 3 were negative. The overall concordance between the assay and carcinogenicity was 72%. The results from the present study were compared with results from the micronucleus test, which were available for 26 of the substances. Results were in agreement for 15 of the

substances, while 8 substances were positive in the present assay and negative in the micronucleus test. It was concluded from this evaluation that the E. coli K-12 DNA repair host-mediated assay detects a number of carcinogens that are negative in the micronucleus test, while detecting most of the compounds that are positive in the latter. The advantages of this test are that differential DNA repair measures a broad spectrum of genetic damage, an in vitro/in vivo comparison is possible with the same test organisms, results can be obtained from various organs and the test is rapid.

38
Jung R, Steinle D, Anliker R. A COMPILATION OF GENOTOXICITY AND CARCINOGENICITY DATA ON AROMATIC AMINOSULPHONIC ACIDS. Food Chem Toxicol 1992; 30(7):635-660.

A review is presented to evaluate existing information on genotoxicity and carcinogenicity testing of various aromatic aminosulphonic acids (AASAs). A great variety of water-soluble azo dyes can form aromatic phenyl-ornaphthyl-aminosulphonic acids by chemical and enzymatic reduction. AASAs are also used as intermediates in the synthesis of azo dyes and azo pigments and can arise as contaminants in the final products. Comparisons have been made with the data available on the corresponding unsulphonated analogues, some of which are known to be genotoxic and/or carcinogenic. The vast majority of the AASAs were conclusively non-mutagenic in the Ames test. In most cases the absence of genotoxicity was also demonstrated with a variety of other test systems in vitro and in vivo. It is concluded that AASAs, in contrast with some of their unsulphonated analogues, generally have no or very low genotoxic and tumorigenic potential.

39
Brambilla G, Martelli A. GRAIN COUNTING IN THE IN VITRO HEPATOCYTE DNA-REPAIR ASSAY. Mutat Res 1992; 272(1):9-15.

The in vitro hepatocyte DNA-repair assay is a widely used useful method in assessing the genotoxic activity of both directly and indirectly acting chemical agents. This article discusses the criteria presently employed in the autoradiographic evaluation of unscheduled DNA synthesis, and suggests that the subtraction of either the average or the highest cytoplasmic grain count,

usually carried out to obtain the net nuclear grain count, may represent a potential source of errors when the test compound is a weakly genotoxic or a nongenotoxic agent. A response can be classified as positive or negative depending on the procedure used to quantitate the cytoplasmic background, and the subtraction of this background from the nuclear count is not founded on a sound theoretical basis because of the following reasons: the different nature of the processes responsible for the generation of nuclear and cytoplasmic grains; and the quantitatively different effect that the test compounds may have on the nuclear and the cytosolic labeling.

40
Knasmuller S, Huber WW, Kienzl H, Schulte-Hermann R.
INHIBITION OF REPAIRABLE DNA-DAMAGE IN ESCHERICHIA COLI K-12 CELLS RECOVERED FROM VARIOUS ORGANS OF NITROSAMINE-TREATED MICE BY VITAMIN A, PHENETHYLISOTHIOCYANATE, OLEIC ACID AND TRIOLEIN.
Carcinogenesis 1992;13(9):1643-50.

The influence of various dietary constituents--phenethylisothiocyanate (PEITC), oleic acid (OA), triolein (TO), and vitamin A (ROL)--on the genotoxic activity of nitrosamines (NDMA, NDELA, NPYR) was investigated. For this purpose differential DNA repair assays with Escherichia coli K-12 strains were performed in vitro and in vivo with mice. Under in vitro conditions (liquid holding), all compounds reduced nitrosamine induced DNA-damage in the indicator bacteria in the dose range 1-10 micrograms/ml, the

ranking order of efficiency being PEITC greater than OA greater than ROL greater than or equal to TO. In animal-mediated assays, acute oral treatment with PEITC (17-150 mg/kg), 2 h before nitrosamine administration, resulted in a marked decrease of nitrosamine genotoxicity in liver, kidneys, lungs and in the blood. Also in other organs (spleen, testes) an increase in differential survival (which serves as a measure for repairable DNA damage) occurred. Biochemical experiments indicated that the antigenotoxic effects of PEITC seen under in vivo conditions were due to inhibition of alpha-hydroxylation of the nitrosamines, whereas ROL and TO appeared not to interfere strongly with this metabolic activation step. Our results indicate that in vitro assays only partly reflect the antigenotoxic properties of the different food constituents in vivo and that animal-mediated DNA

repair assays with E. coli strains are an appropriate approach to study the effects of modifiers of nitrosamine genotoxicity in the living animal.

41
Allavena A, Martelli A, Robbiano L, Brambilla G. EVALUATION IN A BATTERY OF IN VIVO ASSAYS OF FOUR IN VITRO GENOTOXINS PROVED TO BE NONCARCINOGENS IN RODENTS. Teratog Carcinog Mutagen 1992;12(1):31-41.

2-Chlorethanol, 8-hydroxyquinoline, 2,6-toluenediamine, and eugenol, previously found to behave as genotoxins in in vitro systems and as noncarcinogens in rodents, were evaluated for their ability to induce genotoxic effects in vivo. Rats were given a single or two successive doses equal to one-half the corresponding LD50 by gavage, killed at different times after treatment, and examined for the following end points: the frequency of both micronucleated polychromatic erythrocytes in the bone marrow and micronucleated hepatocytes (after partial hepatectomy); the in vivo-in vitro induction of DNA fragmentation, as measured by the alkaline elution technique, and of unscheduled DNA synthesis, as measured by autoradiography, in hepatocyte primary cultures. The two latter end points were also evaluated after in vitro exposure of hepatocytes to log-spaced subtoxic concentrations. 2-Chloroethanol, 8-hydroxyquinoline, and eugenol did not produce effects indicative of genotoxic activity. The same happened with 2,6-toluenediamine, with the exception of a significant increase over controls in the amounts of DNA damage and repair displayed by hepatocyte cultures obtained from rats given two 1/2 LD50 doses separated by a 24 hour interval. The results, which, apart from the above mentioned exception, are in concordance with rodent carcinogenicity results, contribute to underline the role of in vivo short-term tests for the detection of potential genotoxic carcinogens.

42
Knasmuller S, Kienzl H, Huber W, Hermann RS. ORGAN-SPECIFIC DISTRIBUTION OF GENOTOXIC EFFECTS IN MICE EXPOSED TO COOKED FOOD MUTAGENS. Mutagenesis 1992;7(4):235-41.

The induction of organ-specific genotoxic effects of five cooked food mutagens in Swiss albino mice was investigated in microbial animal-mediated assays. The

indicator of the induction of DNA damage was a pair of Escherichia coli K12 strains, differing vastly in repair capacity (uvrB/recA versus uvr+/rec+). All compounds gave positive results in the tested dose range between 2.5 and 40 mg/kg body weight (i.p. administration, exposure time 120 min). 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (MeIQ) and 2-amino-3-methylimidazo[4,5-f]quinoline (IQ) were slightly more genotoxic than 2-amino-3,8-dimethylimidazo[4,5-f]quinoline (MeIQx), 3-amino-1-methyl-5H-pyrido[4,3-b]indole (Trp-P-2) and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine (PhIP) which caused similar effects. The pattern of organ-specific effects was essentially similar for all compounds; genotoxicity was most pronounced in livers and lungs, whereas in kidneys, spleen and testes comparatively lower effects were measured. The results obtained in vivo were compared with data gained in vitro with subcellular organ fractions. Our findings indicate the following. (i) The concentrations required to induce repairable DNA damage in microbial animal-mediated assays are substantially higher than might be expected on the basis of the liquid suspension tests. (ii) The ranking order of the genotoxicity of the various compounds in vitro is similar to that measured in vivo, but the differences in genotoxic potencies are less pronounced in the living animal.

IMMUNOGENICITY

43

Hiestand PC, Graeber M, Hurtenbach U, Herrmann P, Cammisuli S, Richardson BP, Eberle MK, Borel JF. THE NEW CYCLOSPORINE DERIVATIVE, SDZ IMM 125: IN VITRO AND IN VIVO PHARMACOLOGIC EFFECTS. Transplant Proc 1992; 24(4) Suppl 2:31-8.

The search for an immunosuppressive cyclosporine derivative that would surpass the performance of Sandimmune (CyA) in clinical practice has been very difficult. The primary goal, to identify compounds with comparable immunosuppressive properties, has been easy to achieve in vitro using both the classical mixed lymphocyte reaction, and IL-2 production by mitogen-stimulated lymphocytes. The new derivatives, SDZ IMM 125, has been shown to be equipotent to CyA in many in vitro test systems, not to be cytotoxic or cytostatic, and to have a similar ability to suppress production like lymphokine CyA. However, its potential utility as an immunosuppressive agent depends on demonstration of adequate potency in immunologic animal

models.

44
Grossbard ML, Lambert JM, Goldmacher VS, Blattler WA, Nadler LM. CORRELATION BETWEEN IN VIVO TOXICITY AND PRECLINICAL IN VITRO PARAMETERS FOR THE IMMUNOTOXIN ANTI-B4-BLOCKED RICIN. Cancer Res 1992;52(15):4200-7.

Anti-B4-blocked ricin (Anti-B4-bR) is an immunotoxin comprised of the anti-B4 monoclonal antibody and the protein toxin, "blocked ricin.". In blocked ricin, the galactose-binding sites of the ricin B-chain which mediate nonspecific binding to cells are blocked by covalently linked affinity ligands prepared from N-linked oligosaccharides of fetuin. Blocked ricin consists of two species, one with two covalently attached ligands and one with three covalently attached ligands. Although several different lots of Anti-B4-bR had similar IC37 values as determined by in vitro cytotoxicity testing on cultured human cell lines, these lots differed in their in vivo toxicity when administered to patients. Thus, IC37 values alone were not sufficient to predict in vivo toxicity. The degree of cell kill at concentrations of drug that saturated the B4 antigen and murine 50% LD values provide additional parameters that may be predictive of in vivo cytotoxicity. The authors also performed detailed cytotoxicity studies of the ricin species containing two and three covalently attached ligands, respectively. In vitro cytotoxicity testing using these samples revealed that Anti-B4-bR made with blocked ricin containing two covalently attached ligands is capable of depleting five logs of target cells in an in vivo cytotoxicity assay, while Anti-B4-bR comprised of blocked ricin with three ligands can deplete only one log of cells. Log cell kill at antigen saturating concentration, murine 50% LD and biochemical analysis of the compostion of blocked ricin are therefore important considerations for establishing the potential efficacy and safety of Anti-B4-bR.

MUTAGENICITY
45
Sorsa M, Autio K, Abbondandolo A, Carbonell E, Demopoulos N, Garner C, Kirsch-Volders M, Marcos R, Marafante E, et al. EVALUATION OF IN VITRO CYTOGENETIC TECHNIQUES IN NINE EUROPEAN LABORATORIES IN RELATION TO CHROMOSOMAL ENDPOINTS INDUCED BY THREE

MODEL MUTAGENS. Mutat Res 1992;271(3):261-7.

One of the aims of the Commission of European Communities research program on the biomonitoring of human populations exposed to genotoxic environment chemicals has been to compare the sensitivity of DNA and protein adduct detection methods in relation to cytogenetic biomarkers, such as sister chromatid exchanges (SCE), chromosomal aberrations (CA), micronuclei (MN), and point mutations. During the first stage, the objectives of the cytogenetic part of the project have been (1) to correlate the sensitivities of the three basic methodologies in response to the model compounds used; (2) to evaluate the reproducibility of the techniques applied in different collaborating labs; (3) to get some practical experience shipping blood samples between collaborating labs. The three model mutagens responded differently in relation to the cytogenetic end points studied. The trial assured the applicability and reproducibility of the cytogenetic assays.

46
Whong WZ, Stewart JD, Ong T. COMPARISON OF DNA ADDUCT DETECTION BETWEEN TWO ENHANCEMENT METHODS OF THE PHOSPHORUS-32 POSTLABELING ASSAY IN RAT LUNG CELLS. Mutat Res 1992;283(1):1-6.

A 32P-postlabeling analysis is a useful assay system for detecting the covalent binding of mutagens and/or carcinogens to DNA. The detection ability of this system has been tremendously enhanced by the incorporation of butanol extraction or nuclease P1 treatment into the experimental protocol. The sensitivity of adduct detection between these two enhancement methods was compared in vivo and in vitro with 2-aminoanthracene (2AA), 2,4,7-trinitro-9-fluorenone (TNF), and nitrosated coal dust extract (NCDE) using the lung cells of rats. Although, under the conditions tested, both the butanol and the nuclease P1 methods detected DNA adducts caused by all 3 test agents in rat lung cells in vivo or in vitro, a higher adduct detecting ability was found with the butanol enhancement for 2AA and TNF, and with the nuclease P1 enhancement for NCDe. The results suggest that overall the butanol enhancement method is a more sensitive protocol. However, for detecting unknown adduct-forming chemicals, especially when they are present in complex mixtures, both enhancement methods may have to be used.

NEPHROTOXICITY
47
Endou H, Jung KY. MEASUREMENT OF INTRACELLULAR ATP AND CYTOSOLIC FREE CALCIUM FOR IN VITRO NEPHROTOXICITY ASSESSMENT. Rev Pestic Toxicol 1991;1(Pestic Future: Toxicol Stud Risks Benefits):339-48.

The in vitro nephrotoxicity assessment of chemicals was investigated by (1) the effect of HgCl2 and ochratoxin A (OCTA) on cellular ATP content in isolated nephron segments of rats and (2) the effect of HgCl2 and paraquat on angiotensin II (AII)-induced [Ca2+]i transient using early proximal tubules (S1) freshly isolated from rat kidneys. Measurement of cellular ATP and Ca2+ is a sensitive tool for the evaluation of toxic chemicals and also is a reasonable method to study cytotoxic mechanisms.

NEUROTOXICITY
48
Henschler D, Schmuck G, Van aerssen M, Schiffmann D. THE INHIBITORY EFFECT OF NEUROPATHIC ORGANOPHOSPHATE ESTERS ON NEURITE OUTGROWTH IN CELL CULTURES: A BASIS FOR SCREENING FOR DELAYED NEUROTOXICITY. Toxicol In Vitro 1992;6(4):327-335.

Organophosphates have previously been tested for the induction of delayed neuropathy in adult hens. An alternative in vitro test, which avoids the severe suffering caused by the test in hens, has been developed using permanent cell lines from a rat-brain glioma (C-6)from a mouse-brain neuroblastoma (N-18). Addition of dibutyryl cAMP to these cell cultures triggers the development of neurite-like processes; the development of these processes is inhibited by the addition of various organophosphate compounds and this inhibition serves as an indicator of neurotoxicity. 26 compounds with positive results in the in vivo test in hens, and eight analogues with negative results were tested in vitro. An almost perfect correlation between the in vivo and in vitro results was found. The in vitro test is recommended to avoid the need for testing in vivo in hens.

OCULAR TOXICITY
49
Musson DG, Bidgood AM, Olejnik O. AN IN VITRO

COMPARISON OF THE PERMEABILITY OF PREDNISOLONE, PREDNISOLONE SODIUM PHOSPHATE, AND PREDNISOLONE ACETATE ACROSS THE NZW RABBIT CORNEA. J Ocul Pharmacol 1992; 8(2):139-50.

Controversy and ambiguity in the literature concerning the corneal penetration of prednisolone acetate over prednisolone sodium phosphate in NZW rabbits prompted comparative studies using specific chromatographic assays. In vitro, corneal penetration studies were performed in Ussing chambers to compare the permeability and flux of both esters and prednisolone at 0.5%, using a reversed phase HPLC-UV assay. Chromatograms of samples from the receiver chambers showed primarily the presence of prednisolone from both esters; only prednisolone phosphate penetrated the cornea intact. Flux measurements were similar for prednisolone and both salt forms in terms of the metabolite prednisolone. Permeability coeff. calcns. gave the relative comparison: prednisolone acetate > prednisolone > prednisolone sodium phosphate.

50
Ellingson CM, Schoenwald RD, Barfknecht CF, Rao CS, Laban SL. RAPID TOXICOLOGICAL MODEL FOR USE IN ASSESSING OCULAR DRUGS. Biopharm Drug Dispos 1992; 13(6):417-36.

Nonsteroidal antiinflammatory drugs (NSAIDs) were applied to corneas either by in vitro or in vivo methods. The in vitro method involved excising and mounting corneas in a perfusion system at 37 degrees and exposing drug for 2.5 h. The in vivo methods represent either topical administration to the rabbit eye or topical in vivo infusion using a fixed well which permitted a constant concentration (0.05 per cent) to be applied to the eye of anesthetized rabbits for up to 120 min. An overlay grid procedure using scanning electron microscopy (SEM) showed less per cent

endothelial damage with in vivo methods than with the in vitro method of administration, but per cent damage depended on which section was viewed. Damage to the epithelium and endothelium were also assessed by quantitative carboxyfluorescein and Janus green staining and uptake procedures, respectively, following drug exposure by the in vivo infusion method. Qualitative assessment of epithelial and endothelial toxicity can be performed with SEM and transmission electron microscopy (TEM) while vital staining

procedures and the SEM grid procedure can be used to quantitatively assess corneal toxicity. Staining methods, however, possess advantages over SEM and TEM procedures in that they are rapid and do not require laborious preparation. As a result of these characteristics, the vital staining procedures could be used as part of a biopharmaceutical screening technique in evaluating new ophthalmic drugs.

51
Steinsapir KD, Tripathi RC, Tripathi BJ, Ernest JT. INHIBITION OF OCULAR GAMMA GLUTAMYL TRANSPEPTIDASE BY ACETAZOLAMIDE. Exp Eye Res 1992;55(1):179-81.

Acetazolamide inhibited ocular gamma-glutamyl transpeptidase in rats both in vivo and in vitro experiments. Histochemical methodology was used for the detection of the enzyme. In vitro 0.2 and 0.4 mg/mL concetrations of acetazolamide were used, in vivo, 20 mg/kg, i.v. was used.

52
Sina JF, Ward GJ, Laszek MA, Gautheron PD. ASSESSMENT OF CYTOTOXICITY ASSAYS AS PREDICTORS OF OCULAR IRRITATION OF PHARMACEUTICALS. Fundam Appl Toxicol 1992;18(4):515-21.

We have evaluated the use of cytotoxicity assays in vitro as an alternative to predicting ocular irritation potential in animals. Three different measures of cytotoxicity--leucine incorporation into protein, MTT dye reduction, and neutral red uptake--were measured in a presumed target cell, corneal epithelial cells from rabbit, as well as in a nontarget cell, V79 (Chinese hamster lung fibroblasts). An IC50 value was determined for each endpoint in one or both target cells for a series of 27 commercially available compounds and 56 in-house materials from a variety of chemical classes (carbonitriles, imidazoles, substituted benzenes, aromatic acids, peptides, phenols, esters, etc.). Analysis of the data by Spearman rho rank correlation and Pearson's correlation indicated that none of the endpoint-target cell combinations used here accurately predicts in vivo irritation potential for this group of compounds. The MTT dye reduction endpoint gave the best overall correlation, regardless of target cell, but still had a correlation coefficient below -0.5. We conclude that the measurement of cytotoxicity is of

limited value as an alternative assay for the classes of materials studied here.

53
Bagley DM, Bruner LH, De Silva O, Cottin M, O'Brien K AF, Uttley M, Walker AP. AN EVALUATION OF FIVE POTENTIAL ALTERNATIVES IN VITRO TO THE RABBIT EYE IRRITATION TEST IN VIVO. Toxicol In Vitro 1992; 6(4):275-284.

Five alternative techniques, each of which had been successfully used by one of the participating companies, were evaluated in the assessment of the eye-irritation potential of 32 samples. The 32 samples included chemical ingredients and preparations from household cleaning product, personal care, and cosmetic categories. Historical data from rabbit eye irritation tests in vivo existed for each sample; it was therefore not necessary to carry out any tests in vivo as part of this evaluation exercise. The five alternative methods used were the silicon microphysiometer test, the Microtox test, the neurtral red uptake assay, the chorioallantoic membrane vascular assay (CAMVA) and the hen egg test-chorioallantoic membrane assay (HETCAM). Three of the 5 assays were conducted in two laboratories, allowing an interlaboratory comparison of performance to be made. The results demonstrated that for the materials tested, all of the assays show some promise as alternative methods to the rabbit eye test in vivo in the prediction of eye irritation, and that the reproducibility of results of those techniques carried out in two laboratories was very good. The results from 14-day and 10-day CAMVA assays were virtually identical. It is recommended that a larger-scale validation exercise be carried out to demonstrate the ultimate usefulness of these alternative procedures.

PULMONARY TOXICITY
54
Ben-Jebria A, Marthan R, Savineau J-P. EFFECT OF IN VITRO NITROGEN DIOXIDE EXPOSURE ON HUMAN BRONCHIAL SMOOTH MUSCLE RESPONSE. Am Rev Respir Dis 1992; 146(2):378-382.

The aim of this study was to develop an in vitro system in which an isolated bronchus from human lung was exposed, during 30 min, to a constant flow of either air or nitrogen dioxide (NO2), and to examine

subsequently the contractile response of airway smooth muscle rings to carbachol, histamine, and substance P. Two proximal bronchi were mounted in an organ bath, perfused externally with Krebs-Henseleit solution and ventilated with clean air, 1.0 or 2.0 ppm NO2. The exposed bronchi were then cut into rings and mounted in a computerized organ bath system. Contractile response to agonists were measured isometrically. In each ring, a cumulative concentrations response curve was obtained to the desired agonist. We found that in vitro exposure of human lumen bronchus to a constant flow of air did not alter the contractility of the smooth muscle. Whereas in vitro exposure of the bronchus to 1.0 ppm NO2 did not significantly increase the efficacy or the potency of carbachol, exposure to 2.0 ppm NO2 increased airway smooth muscle contractions in response to carbachol, histamine, and substance P. The results indicated the experimental preparation is well suited to study the respiratory toxicity of inhaled pollutants in order to understand further the mechanisms underlying toxicant-induced airway hyperresponsiveness.

55
Hsu MT, Dimaio M, Reiss OK, Ciurea D, Gil J. A NOVEL SYSTEM FOR THE CULTURE OF HUMAN LUNG: LUNG DEVELOPMENT AND THE RESPONSE TO INJURY. Am J Physiol 1992;263(3) Pt 1:L308-16.

Existing methods of fetal lung organ culture are complicated and require special skills. With the use of a polyester-based plastic sheet, we have developed a simpler human fetal lung organ culture that is viable for 6 wk. This novel method permits the study of growth and differentiation, pulmonary surfactant secretion, and the response of human lung tissue to injury in vitro. Microscopic study of the fetal lung before culturing revealed round epithelial tubules, lined by glycogen-rich columnar cells and a thick cellular interstitium. After 1 wk in culture, morphological examination showed the development and expansion of alveolar saccules and thinning of the interstitium; type I and II pneumocytes as well as fibroblasts and myofibroblasts were present. Lipid analysis of the tissues, 2 wk after the initiation of the culture, demonstrated a high percentage of dipalmitoyl phosphatidylcholine characteristic of pulmonary surfactant. Treatment of the organ culture with asbestos fibers induced type II cell hyperplasia, increased numbers of collagen fiber bundles within the

interstitium, and the accumulation of multi-lamellated surfactant material within the alveolar lumens. It was concluded that this organ culture system is suitable for studying lung growth, development, and injury in human tissue.

56
Centra M, Ratych RE, Cao GL, Li J, Williams E, Taylor RM, Rosen GM. CULTURE OF BOVINE PULMONARY ARTERY ENDOTHELIAL CELLS ON GELFOAM BLOCKS. FASEB J 1992; 6(12):3117-21.

Conventional methods of endothelial cell culture on monolayers and beads require enzymatic digestion, traumatic scraping, or centrifugation to transfer cells to other experimental systems. Gelfoam, a porous gelatin block, not only supports the growth of bovine pulmonary artery endothelial cells but also allows the rapid transfer of cell-laden blocks from one experimental system to another with minimal intervention. This property has been shown to be especially useful for the rapid fixation of endothelial cells for microscopy using standard histologic methods. Histology confirmed that the trabecular nature of the substrate allows endothelial cells to line the interstices of the sponge matrix and grow in a configuration that simulates the appearance of the endothelium in small vessels and capillaries. To facilitate cell counting, the Gelfoam matrix was rapidly removed by the addition of 0.05 mg/ml collagenase, a concentration that interfered minimally with the assay for cellular protein concentration. The data demonstrate that Gelfoam is a suitable support growth matrix for the in vitro culture of bovine pulmonary artery endothelial cells.

57
Thoren SA. CALORIMETRY: A NEW QUANTITATIVE IN VITRO METHOD IN CELL TOXICOLOGY: A DOSE/EFFECT STUDY OF ALVEOLAR MACROPHAGES EXPOSED TO PARTICLES. J Toxicol Environ Health 1992;36(4):307-318.

A short-term toxicological test has been developed using a calorimetric method. The metabolic activity, observed as the heat exchange rate, was monitored from alveolar rabbit macrophages in monolayers exposed to different metal and non-metal particles. Calorimetric activity indices and viability indices were introduced, from which toxic effects could be assessed. Manganese

dioxide particles were found to be cytotoxic. In contrast, titanium dioxide particles seemed to be harmless. The results were in accordance with the cell survival found by use of a fluorescein ester staining method and measured by an image analyzer. Toxic effects from quartz in the form of increased metabolic activity of exposed cells could be detected by the calorimeter in contradiction to the use of the image analyzer. This latter result supports the hypothesis that silica particles cause chronic modification of the macrophage function and that this change in the alveolar macrophage function may be the first of a series of processes leading to pulmonary fibrosis.

REPRODUCTIVE TOXICITY

58

Bara M, Guiet-Bara A, Durlach J. A NEW METHOD OF IN VITRO PRESCREENING EVALUATION OF THE RELATIONSHIP BETWEEN TOXIC AND COMMON METAL IONS. Methods Find Exp Clin Pharmacol 1992;14(4):311-14.

The human amniotic membrane, an asymmetric and nonexcitable epithelium with sites differently situated on the fetal and maternal sides, may be considered a model for investigating the relationship between toxic and common metal ions. The method is based on the observation of the ionic transfer across the amnion, estd. by measuring the total ionic conductance Gt from the mother to the fetus and from the fetus to the mother. It is important to note that opposite effects between two ions are not necessarily correlated with antagonism; indeed, pollutants decrease ionic conductance Gt and Mg increases it, but Mg is not an antagonist of all pollutants. To define antagonism between two ions, the Dixon curves theory should be applied. At pharmacological doses, there is competitive inhibition (specific antagonism) between Mg and Cd, Zn and Cd, Ca and Cd, and Mg and Pb, and noncompetitive inhibition between Mg and Hg. This method may rapidly indicate a membrane interaction between common and toxic metals.

59

Omarini D, Barzago MM, Aramayona J, Bortolotti A, Lucchini G, Bonati M. THEOPHYLLINE TRANSFER ACROSS HUMAN PLACENTAL COTYLEDON DURING IN VITRO DUAL PERFUSION. J Med (Westbury, N. Y.) 1992;23(2):101-16.

In vitro placental perfusion is widely used to

investigate the placental transfer of endogenous compounds and, to a lesser extent, that of drugs. The aim of this study was to assess the suitability and reliability of such in vitro systems for application on drug placental transfer studies. The authors investigated the time course of theophylline (TH) transfer, a drug frequently used in the perinatal period. Eight experiments were performed with maternal and fetal circuits maintained in an open system, perfusing placentas for 160 min with Earle's enriched bicarbonate buffer containing two test substances, antipyrine (AP), (80 mg/L) and creatinine (CR), (150 mg/L), and the tool drug TH (15 mg/L). All substances equilibrated in the system with time proportional to the chemical-physical characteristics of each compound, being the time required to reach the steady state 5 to 12 min. for AP, 12 to 31 min. for CR and 10 to 35 min. for TH. AP and CR clearances were 2.94 .+-. 0.33 and 0.83 .+-. 0.26 mL/min, respectively. The transfer profile of TH was similar to that of AP and its clearance was 2.39 .+-. 0.37 mL/min, with a clearance index of 0.80 .+-. 0.11. Transfer percentages of TH are in agreement with in vivo values for both humans and animals, and with results obtained during in situ placental perfusion in the rabbit. Physiological conditions and biochemical properties of the tissue were well maintained throughout perfusion. The findings support the reliability of this technique to study transplacental passage of drugs, and the relevance of such a model to obtain information concerning potential therapeutic or toxicological effects of drugs during the last trimester of pregnancy.

TERATOGENCITY

60

Welsch F. IN VITRO APPROACHES TO THE ELUCIDATION OF MECHANISMS OF CHEMICAL TERATOGENESIS. Teratology 1992; 46(1):3-14.

A review with 31 references on some of the contributions that in vitro methods have made toward understanding mechanisms of chemical teratogenesis. Emphasis is given to the painstaking and time consuming nature of approaches required to elucidate mechanisms. The examples considered are cyclophosphamide, 2-methoxyethanol, and retinoids. Some of the newer methods that take advantage of the recent advances in molecular biology and analytical chemistry have been applied to studies on teratogenic mechanisms.

Prospects for the 1990s are excellent and promise more rapid progress than during the past decade toward unraveling the mysteries of normal development biology. That knowledge in turn should be immediately applicable for investigations on developmental toxicant-induced abnormal development.

61
Bechter R, Terlouw GD C, Tsuchiya M, Tsuchiya T, Kistler A. TERATOGENICITY OF AROTINOIDS (RETINOIDS) IN THE RAT WHOLE EMBRYO CULTURE. Arch Toxicol 1992;66(3):193-7.

Structural modifications of the carotinoid mol. RO 13-7410 led to a difference in the teratogenic potencies of more than five orders of magnitude in mice in vivo and in micromass cultures of rat embryonic limb bud cells (Kistler et al., 1990). Five of these retinoids were selected and tested in rat whole embryo culture to determine the suitability of this in vitro test system for the identification of potentially non-teratogenic derivatives among this class of chemicals. The highest concentations of the compounds with no effects (NOAEL) on general conceptus growth, on differentiation and on the frequency of dysmorphogenic embryos in vitro were compared with the lowest effective teratogenic doses in vivo (LOAEL) or with the concentrations leading to 50% inhibition of limb bud cell differentiation (IC50) in vitro. NOAEL's for the parameters of conceptus development ranged from 10-5 mug/mL (0.03 nM) to 10 mug/mL (28.7 muM) for the compounds tested. These correlated very well with LOAEL and IC50 (R >0.95). The types of dysmorphogenesis in vitro were those typical for retinoids, and for the most part resembled the malformations found in vivo. It was concluded that the whole embryo culture system is a useful tool for the preliminary testing of retinoids.

62
Kucera P, Honegger P, Zijlstra J, Schmid B. THREE IN-VITRO TOXICITY-TERATOGENICITY TEST SYSTEMS VALIDATED BY USING TWELVE IDENTICAL CODED COMPOUNDS. 24TH Annual Meeting of the Swiss Societies for Experimental Biology (usgeb/ussbe), Basel, Switzerland, March 19-20, 1992. Experientia (Basel) 1992;48(Abstr):A33.

No abstract.

MISCELLANEOUS

63

Bragadin M, Argese E, Nicolli A, Bernardi P. A SIMPLE IN VITRO TEST TO MONITOR TRACE METAL TOXICITY IN AQUEOUS SAMPLES. Environ Technol 1992;13(8):779-784.

We have studied the effect of test toxicants on electron transfer along the rotenone-insensitive NADH-cytochrome b5 reductase of the outer mitochondrial membrane, using oxidized cytochrome c as the electron acceptor in a simple spectrophotometric assay. We show that this reaction is extremely sensitive to trace metals of environmental concern, detecting reliably as little as 10 nM Hg2+. The assay is reproducible, simple, fast and inexpensive, compares favorably with fish acute toxicity tests, and may become useful in the routine monitoring of waters for the presence of this class of pollutants.

64

Harms HH. IN VITRO SYSTEMS FOR STUDYING PHYTOTOXICITY AND METABOLIC FATE OF PESTICIDES AND XENOBIOTICS IN PLANTS. Pestic Sci 1992;35(3):277-281.

Plant cell cultures have been used for ecotoxicological evaluations of pesticides and xenobiotics and results are compared with those using intact plants grown under aseptic conditions. Both plant test systems were able to metabolize the compounds by common metabolic pathways. Qualitatively, the metabolites were the same in both systems. However, using cell suspension cultures, the results may be obtained more quickly with less analytical expense. Such cultures are therefore useful systems for obtaining rapid evidence of the ecotoxicological behaviour of chemicals in plants.

65

Xue K, Ma G, Wang S, Wang Y. NUCLEAR ANOMALY TEST IN HUMAN LYMPHOCYTES IN VITRO. Zhongguo Yaoli Xuebao 1992;13(5):464-7.

To assess the usefulness and the sensitivity of the nuclear anomaly test in human lymphocytes, in vitro human whole blood was treated with various concentrations of mitomycin C (MMC), thiotepa, and bimolane. After the blood samples had been stored at 37.degree. for 17-18 h, smears of isolated lymphocytes were made. The nuclear anomalies (micronuclei, irregular, karyorrhectic, and pyknotic nuclei) were

measured. The concentration-response relationship and the min. sensitive concentration of nuclear damage indexes to the test mutagens were analyzed. The results showed that all 3 drugs induced a concentration-dependent increase of nuclear anomalies except pyknotic nucleus in lymphocytes. The most sensitive index of nuclear damage was the micronucleus assay. The karyorrhectic assay was as sensitive to MMC and bimolane as the micronucleus assay. The irregular nucleus assay and the complex nuclear anomaly assay were less sensitive. However, the correlation between

concentration and complex nuclear anomalies was the best among various indexes of nuclear damage. Therefore, the in vitro nuclear anomaly test in lymphocytes of human whole blood could be used to evaluate genotoxic effects of chemicals.

66
Bara M, Guiet-Bara A, Durlach J. A NEW METHOD OF IN-VITRO PRESCREENING EVALUATION OF THE RELATIONSHIP BETWEEN TOXIC AND COMMON METAL IONS. 13th Allerheiligengespraech (All Saints' Day Conference) on Pharmacokinetics of Electrolytes, Bobenheim, Germany, October 31-November 1, 1991. Exp Clin Pharmacol 1992;14(4):311-314.

No abstract.